

Gross embryology of a monotrysian heteroneuran moth, *Stigmella castanopsiella* Kuroko (Nepticulidae, Lepidoptera) and its phylogenetic significance

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Abstract The formation of the germ disk and embryonic membranes and changes in the shape of the developing embryo of a monotrysian heteroneuran moth, *Stigmella castanopsiella*, were described for evaluating the systematic position of the family Nepticulidae within the Lepidoptera from the embryological standpoint. The mode of the embryonic development of this species, as a whole, was very similar to that of higher ditrysian species, but differed from that of primitive homoneuran species. In particular, the amnion and serosa of *S. castanopsiella* were suggested to be formed in the same fault-typed manner as that of ditrysian species, and the embryo of this species developed under the immersed condition in the yolk as commonly observed in ditrysian eggs. These embryonic characters are considered as apomorphic ones shared by the Nepticulidae and Ditrysia, and consequently these embryological findings added further weight to the view that the Heteroneura are monophyletic.

Key words Embryo, embryology, *Stigmella*, Nepticulidae, Monotrysia, Heteroneura, phylogeny.

Introduction

According to the condition of the wing venation, the Lepidoptera can be divided into two groups, the Homoneura and Heteroneura, although the former is not considered as a monophyletic taxon. On the basis of the structure of the female genitalia, the latter is further subdivided into two groups, the monotrysian heteroneuran families (formerly called Monotrysia) and the monophyletic taxon Ditrysia having the genital apertures of the ditrysian condition. The family Nepticulidae, to which *Stigmella castanopsiella* belongs, is one of the former families. The monotrysian heteroneuran families can be grouped into four monophyletic superfamilies, *i. e.*, the Incurvarioidea, Nepticuloidea, Palae-phatoidea, and Tischerioidea. Davis (1986) considered Tischerioidea to represent the sister group of the Nepticuloidea, while Nielsen (1991) proposed that the Palae-phatoidea+Tischerioidea are the sister group of the Ditrysia; thus the phylogenetic relationships among these taxa remain unsolved.

The Ditrysia are the most advanced, higher Lepidoptera whose embryogenesis is well known. In recent years, the embryonic development of the primitive homoneuran Lepidoptera, such as *Neomicropteryx*, *Eriocrania*, *Endoclita*, and *Mnesarchaea*, also became evident in a series of our works (Ando and Tanaka, 1976, 1980; Ando and Kobayashi, 1978; Kobayashi and Ando, 1981, 1982, 1983, 1984, 1987; Kobayashi and Gibbs, 1995; Kobayashi *et al.*, 1981; Tanaka *et al.*, 1985), and the embryological information has made possible to evaluate the phylogenetic relationships among the Ditrysia and these primitive Lepidoptera (Kobayashi and Ando, 1988). From the comparative embryological standpoint, it is an interesting subject of study to know whether the mode of embryonic development of the monotrysian heteroneuran species is similar to that of

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the Ditrysia or the Homoneura. However, the embryological information on these species is almost lacking, although the unpublished embryological data of *S. castanopsiella* have appeared fragmentarily in our article cited above. In the present paper, I will describe the gross embryology of *S. castanopsiella*, and discuss its phylogenetic significance.

Materials and Methods

Stigmella castanopsiella is a minute moth whose larva is a leaf-miner. Its eggs are deposited singly on the upperside of the leaves of the host plant, *Castanopsis cuspidata* Schotky, in late May. In natural conditions, the larvae hatch out in mid-October of the year (Hisai, 1982); thus the egg period is about 135 days (4.5 months). The eggs undergo summer diapause dependent on a high temperature in summer (Kino, 1981).

The eggs at various developmental stages were collected at the National Park for Nature Study in Tokyo, Japan, from May to October of 1982. Most of them were fixed immediately in alcoholic Bouin's solution or FAA at room temperature, mounted in paraffin, sectioned at 7 μ m, and stained with Delafield's hematoxylin and eosin. To observe the external shape of the embryos and its changes in living state, several eggs were mounted in liquid paraffin, and observed under a differential interference microscope.

Observation

(1) Egg

The newly laid eggs of *S. castanopsiella* are whitish yellow, and flattened ellipsoidal, about 0.4 mm in length, 0.3 mm in width, and 0.06 mm in thickness. The egg length and width are constant throughout the egg period, while the thickness increases to about 0.1 mm as development proceeds. The chorion is dense and almost transparent, and of about 10 μ m in thickness. At the anterior pole of the egg (either end of the apse line), four micropylar canals are observed.

(2) Formation of blastoderm, germ rudiment and embryonic membranes

At 3 days after oviposition, most of the cleavage nuclei or energids reach the periphery of the yolk; consequently a thin blastoderm forms at the egg periphery. A few energids remain in the yolk to become the primary vitellophages.

At 5 days, the blastoderm cells on the ventral side of the egg (side attached to the leaf) divide actively by mitoses and form a slightly thick germ disk, whereas those on the dorsal side (extraembryonic area) hardly cleave (Fig. 1). The cells of the germ disk then become columnar and congregate to the mid-ventral part of the egg, forming a small and circular germ rudiment. At 7 days, the germ rudiment slightly sinks into the yolk. Although the exact process of the formation of the serosa and amnion was not observed in the present study, as the germ rudiment sinks into the yolk, the extraembryonic area is probably cut off along the margin of the germ rudiment, and spreads over it to become the serosa (Fig. 2). The amnion seems to proliferate from the margin of the germ rudiment after completion of the serosa (Fig. 3). The yolk cleavage occurs after completion of the germ rudiment, and the yolk is divided into many spherical masses each enclosed with a delicate yolk membrane (Fig. 4). The eggs then enter summer diapause, that is, no further changes occur in the shape and size of the germ rudiment until early in September.

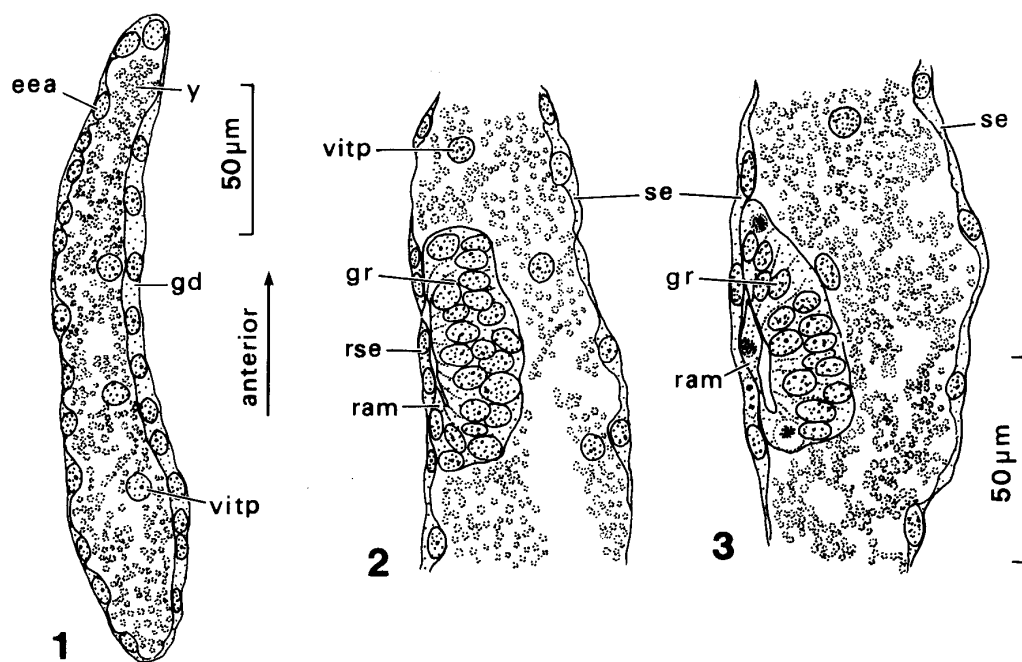
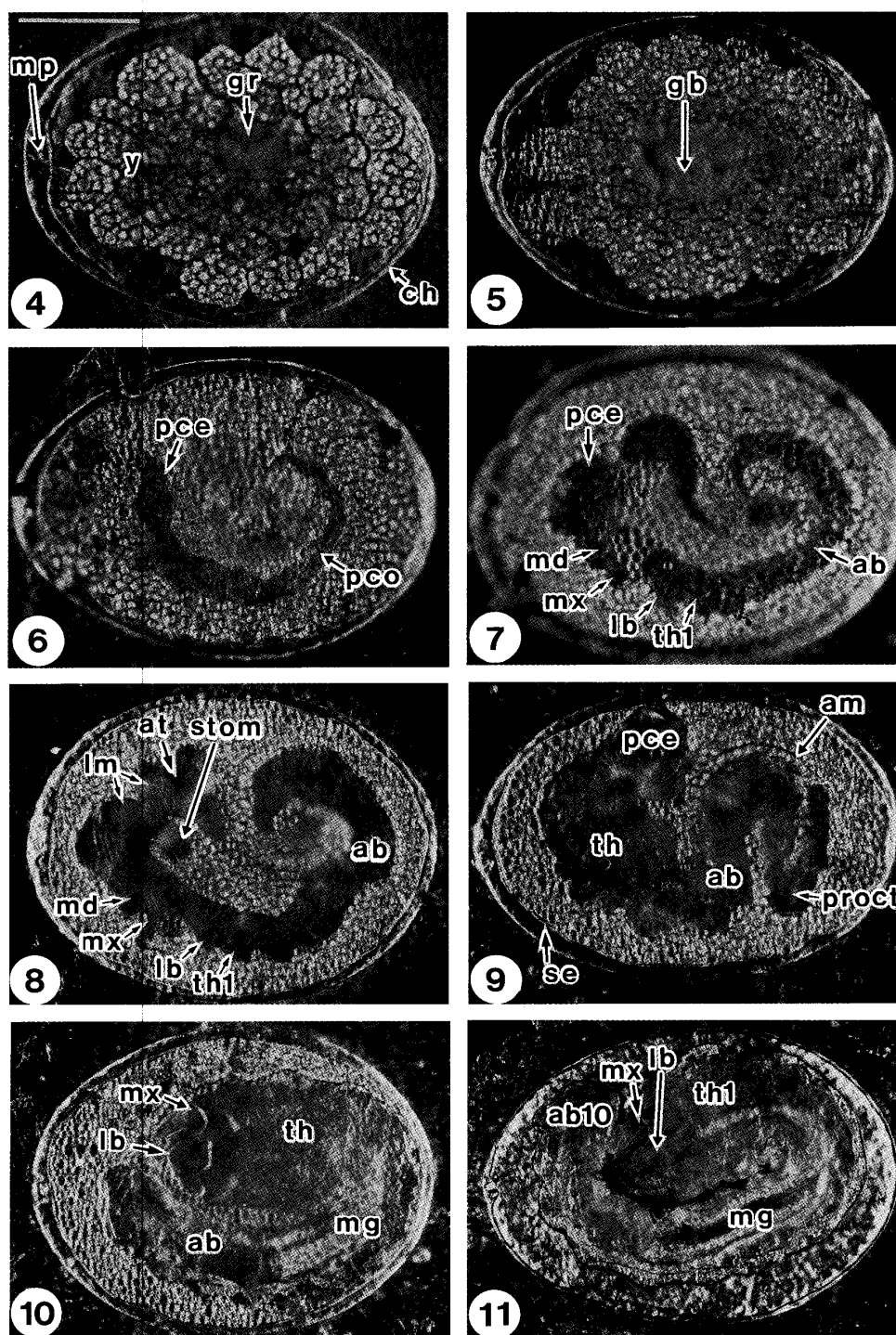


Fig. 1. Longitudinal section of 5-day egg of *Stigmella castanopsiella*. Fig. 2. Cross section of 7-day egg of *S. castanopsiella* showing germ rudiment and formation of serosa and amnion. Fig. 3. Cross section of 7.5-day egg of *S. castanopsiella* showing germ rudiment and completed serosa (eea: extraembryonic area, gd: germ disk, gr: germ rudiment, ram: rudimentary amnion, rse: rudimentary serosa, se: serosa, vitp: vitellophage, y: yolk).

(3) Changes in external shape of embryo

Early in September, the germ rudiment comes out of diapause, and elongates antero-posteriorly to become the germ band or embryo curled dorsally (Fig. 5). The germ band then increase in length and width, and a bilobed protocephalon and a slender protocorm become apparent (Figs 6, 7). In sections, the germ band at this stage is observed to be immersed completely into the yolk. In mid-September, the labral lobes, antennal rudiments, rudimentary mandibular, maxillary, and labial appendages appear in the cephalognathal region (Fig. 8). The stomodaeal opening is observed in the mid-posterior part of the protocephalon. However, the thoracic appendages are rudimentary and obscure. The posterior end of the abdomen strongly curls dorsally. As the germ band grows, its cephalognathal region twists ventrally, so that the ventral side of the cephalognathal region faced toward the attached surface of the egg. Meanwhile the yolk membranes enclosing yolk spherules gradually disappear, thus the yolk cleavage breaks down completely (Fig. 9). In late September, the embryo accomplishes revolution (Fig. 9); that is, the posterior segments of the embryonic abdomen are first turned ventrally causing the abdominal end to shift anteriorly, and finally the end reaches the level of anterior end of the head. This process proceeds in the yolk, hence many yolk droplets are observed around the embryo even after the completion of revolution. The amnion and serosa do not rupture at the time of or after revolution, and consequently the secondary dorsal organ is not formed.

Soon after revolution, all gnathal appendages project forwards and the dorsal closure of the embryo is completed. Several days after revolution, the mandibles become brown and the embryo begins to eat surrounding yolk materials left outside the embryo after the dorsal closure (Fig. 10). Peristalsis of the midgut is frequently observed accompany-



Figs 4-11. Embryonic development of *Stigmella castanopsiella*. 4. 7-day egg showing small germ rudiment. 5. About 100-day egg showing embryo just aroused from summer diapause. 6. About 105-day egg showing elongated embryo. 7. About 110-day egg showing formation of appendages in cephalognathal region. 8. About 115-day egg showing completion of cephalognathal appendages. 9. About 125-day egg showing revolution of embryo. 10. About 130-day egg showing ingestion of residual yolk around embryo. 11. About 135-day egg showing full-grown embryo just before hatching (ab: abdomen, ab10: 10th abdominal segment, am: amnion, at: antenna, ch: chorion, gb: germ band, gr: germ rudiment, lb: labial segment, lm: labral lobes, md: mandibular segment, mg: midgut, mp: micropyle, mx: maxillary segment, pce: protocephalon, pco: protocorm, proct: proctodaeum, se: serosa, stom: stomodaeum, th: thoracic segment, th1: prothorax, y: yolk). Scale, 100 μ m.

ing the ingestion of the yolk. In mid-October, soon after the residual yolk is eaten by the embryo (Fig. 11), the full-grown embryo or the first instar larva gnaws off the chorion with the mandibles, and eats into the tissue of the host plant on which the egg is deposited. The amnion and serosa seem to be ingested by the embryo shortly before hatching.

Discussion

The remarkable feature in the early embryonic development of *S. castanopsiella* is the formation of a very small and circular germ rudiment by the marked congregation of the broad germ disk at the ventral side of the egg. In the ditrysian lepidopteran eggs, the germ disk is also broad at the time of its formation, but it is immediately cut off from the extraembryonic area, and slightly sinks into the yolk. The germ disk then becomes somewhat compact to form a large, cup-shaped germ rudiment. Thus the congregation of the germ disk to form a small germ rudiment as observed in *S. castanopsiella* does not occur at the egg periphery. Since a similar small germ rudiment is formed in the eggs of *Neomicropteryx* and of *Endoclita*, this feature is thought to be primitive, although in these genera the germ rudiment is formed by the deep invagination of the germ disk (Ando and Tanaka, 1976, 1980; Kobayashi and Ando, 1982). In this respect, therefore, *S. castanopsiella* is different from the ditrysian species, and its eggs are regarded as retaining the ancestral condition of the ditrysian eggs.

The increase in the egg volume of *S. castanopsiella* is considered as another primitive character, because this phenomenon occurs in many hemimetabolous insects, and in the Lepidoptera it has been observed in the homoneuran *Neomicropteryx* and *Eriocrania* and the monotrysian heteroneuran *Nemophora raddei* (e. g. Kuroko, 1961) but has not been observed in all ditrysian eggs. The eggs of *Neomicropteryx* and *Eriocrania* have hydropyle cells which probably function as absorbing water from the outside and thus result in the increase in the egg volume. In *S. castanopsiella*, however, the cells were not found.

The exact process of the formation of embryonic membranes in *S. castanopsiella* was not observed, but the membranes seem to be formed in the same manner as observed in the ditrysian eggs; that is, after the germ disk is cut off from the extraembryonic area, the serosa is completed by the spread of the extraembryonic area over the ventral surface of the germ disk, and then the amnion is formed independently by the reflexed extension of the edge of the germ disk. This manner of embryonic membrane formation, called a fault type (Kobayashi and Ando, 1988), is unique in insect embryogenesis, thus supposed a synapomorphic character shared by the Nepticulidae and Ditrysia.

So far as it is observed externally, the mode of the embryonic development of *S. castanopsiella* after summer diapause is very similar to that of the ditrysiens, such as *Chilo suppressalis* (e. g. Okada, 1960) and *Epiphyas postvittana* (e. g. Anderson and Wood, 1968), of which eggs are also flattened ellipsoid like those of *S. castanopsiella*, and no essential differences are detected among them. In these flattened eggs, as well as other spherical, upright ditrysian eggs, the embryo develops under the completely immersed condition in the yolk until just before hatching. The yolk remaining on the periphery of the egg is eaten by the full grown embryo. In the homoneuran species, on the other hand, the embryo assumes a nearly superficial position throughout development, although the posterior abdominal region of the young embryo of *Neomicropteryx* is temporarily immersed in the yolk. The superficial position of an embryo is widely distributed throughout holometabolous insects and is thus regarded as a generalized or plesiomorphic character, whereas the immersed condition is considered as a highly specialized or

apomorphic one only observed in the Nepticulidae and Ditrysia; thus forming another synapomorphy of these taxa.

In conclusion, although there are some primitive characters, such as the formation of the small germ rudiment and the increase in the egg volume, the mode of the embryonic development of *S. castanopsiella*, as a whole, closely resembles that of the Ditrysia, but differs from that of the homoneuran *Neomicropteryx*, *Eriocrania*, and *Endoclita*. Furthermore, the separation of the Heteroneura from the homoneuran taxa and the resultant monophyly of the Heteroneura will be supported by the possible two embryonic synapomorphies or autapomorphies; (1) the fault-typed formation of embryonic membranes, (2) the embryo develops under the immersed condition in the yolk. However, in order to resolve the problem of the phylogeny of the monotrysian Heteroneura and their relationship with the Ditrysia, we need to make further observations on the embryonic development of other taxa, for example, the members of the Incurvarioidea, Palaeophatoidea, and Tischerioidea.

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摘 要

シイモグリチビガの胚発生とその系統学的意義 (鱗翅目: モグリチビガ科) (小林幸正)

近年, コバネガ, スイコバネガ, およびコウモリガなどのいわゆる原始的な蛾類の胚発生が明らかにされるにつれ, これらの蛾類と胚発生がよく知られている二門類との系統関係が, 発生学的にも論じられるようになった。しかし, モグリチビガ科の属するいわゆる単門類 (Monotrysia) の胚発生についてはまとまった報告はない。本報では, この科に属するシイモグリチビガ *Stigmella castanopsiella* の胚発生の概要を生卵および固定卵の観察に基づき記載した。

本種の卵は5月に食樹のスタジイの葉の表面に1個ずつ産下される。卵は長径0.4 mm, 短径0.3 mm, 厚さ0.06 mmの平たい楕円体で, 発生が進むにつれて, 厚さのみが0.1 mmに増大する。卵期間は約4.5ヶ月で, 卵は夏の胚休眠を経て10月に孵化する。胚盤は産卵後3日目に完成し, 5日目に卵腹面に胚盤が形成される (Fig. 1)。胚盤はやがて卵腹面の中央部に集中し円盤状の胚原基となるが, この際, 胚原基と胚外域との境界部が切れて, 後者が前者の腹面を覆うように伸展して漿膜がまず完成し, 羊膜はこれとは独立に形成されることが示唆された (Figs 2, 3)。その後, 胚原基は細長い小さな胚帯 (胚) となり, この状態で9月初旬まで休眠に入る (Figs 4, 5)。休眠から覚めた胚は卵黄中に沈んだ状態で急速に成長し, 胚の姿勢転換を経て初令幼虫が完成する (Figs 6-11)。漿膜と羊膜は姿勢転換後も破れず存在し, したがって二次背器は形成されない。また, 卵黄は姿勢転換後も胚の外側に残存し, これは完成した初令幼虫によって孵化するまでに中腸内に飲み込まれる。

本種の卵は, 小さな胚原基が形成される点や卵の体積が増大する点で原始的な性質を残しているが, 胚発生の様式は全体としてコバネガやコウモリガなどの原始的な蛾の発生様式よりも二門類のそれに近似している。とりわけ, 胚原基と胚子膜の形成様式が二門類に特有とされた断層型に属すると思われる点や, 孵化直前まで胚が卵黄中に沈んだ状態で発生する点は, 本種と二門類の発生における固有特化形質と見なすことが出来る。したがって, 今回得られた発生学的知見は, 本種を含むいわゆる単門類と二門類が単系統群の Heteroneura を構成するという見解と矛盾しない。

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